Two Species of Dermatan Sulfate Proteoglycans with Different Molecular Sizes from Newborn Calf Skin

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Two species of dermatan sulfate-proteoglycans (DS-PGs) were isolated from calf skin. The first species, PDS-H (high-molecular-weight proteodermatan sulfate), contains the core protein with a molecular weight of either about 55,000 or 53,000. Both the core proteins are capable of binding to concanavalin A (Con A). The second species, PGs-L (low-molecular-weight proteoglycan containing dermatan sulfate and/or chondroitin sulfate), contains a core protein of M_r = 20,000 that did not bind to Con A. Tryptic peptide mappings revealed that M_r = 55,000 core protein and M_r = 53,000 core protein were of the same origin. However, the tryptic peptides and the amino acid composition of PGs-L core protein were completely different from those of PDS-H core proteins. The polyclonal antibodies against M_r = 55,000 core protein reacted with both the core proteins of M_r = 55,000 and M_r = 53,000 but not with the core protein from PGs-L. The DS was found to be the only glycosaminoglycan component of PDS-H. That is, the glycosaminoglycan from PDS-H was composed of 46% iduronosylhexosamine units and 54% glucuronosylhexosamine units, while the glycosaminoglycan of PGs-L was composed of 30% iduronosylhexosamine units and 70% glucuronosylhexosamine units. *J Invest Dermatol 87:221–226, 1986*

Various kinds of proteoglycans (PGs) exist specifically in tissues together with different extracellular molecules. Glycosaminoglycans were reported to be tightly associated with collagen [1–3], to affect collagen fiber formation in vitro [4,5], and to have the function of stabilizing collagen fibrils in vivo [6]. Proteoglycans have been shown to bind to specific amino acid residues in soluble collagen [7] and to induce conformational changes in the molecule [8]. These results suggest that PGs play a role in determining the macromolecular architecture of the extracellular matrix. Dermatan sulfate-rich PG coexisting in collagen fiber at the D-band was observed in rat tail-tendon collagen by electron microscopy [9]. Dermatan sulfate which is covalently bound with M_r = 55,000 core protein was extracted by neutral salt from newborn calf skin, though a major portion or 70% of DS in the tissues was associated in neutral salt-insoluble materials. Chondroitin sulfate was also found in neutral salt-insoluble collagen fractions [10]. In bovine aorta, CS and DS have been reported to be attached to the same core protein as copolymeric CS-DS-PGs [11]. In human aorta [12] and in chick aorta [13], CS and DS have been observed in separate PG monomers. To investigate the relation between proteodermatan sulfate (PDS) and the collagen fiber, PDSs in neutral salt-insoluble collagen were extracted with 4 M guanidinium chloride. The structural characterization of the extracts is described.

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Abbreviations:
Con A: concanavalin A
CS: chondroitin sulfate
C4S: chondroitin 4-sulfate
ΔDi-OS: 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose
ΔDi-4S: 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose
ΔDi-6S: 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose
DS: dermatan sulfate
HA: hyaluronic acid
HP: heparin
HS: heparan sulfate
PBS: phosphate-buffered saline
PCS: proteochondroitin sulfate
PDS: proteodermatan sulfate
PDS-H: high-molecular-weight proteodermatan sulfate
PG: proteoglycan
PGs-L: low-molecular-weight proteoglycan containing dermatan sulfate and/or chondroitin sulfate
SDS: sodium dodecyl sulfate
TLC: thin-layer chromatography

MATERIALS AND METHODS

Materials Sepharose CL-4B, DEAE-Sephadex A-50, and concanavalin A (Con A)-Sepharose 4B gels were purchased from Pharmacia Fine Chemicals, Uppsala; Ultrogel AcA-44 was from LKB Industrie Biologique Française, Paris; chondroitinase ABC (EC 4.2.2.4), chondroitinase AC (EC 4.2.2.5), *Streptomyces* hyaluronidase (EC 4.2.2.1), endo-β-N-acetylglucosaminidase H (EC 3.2.1.96), hyaluronic acid (HA), DS, chondroitin 4-sulfate (C4S), heparan sulfate (HS), heparin (HP), and unsaturated disaccharide units ΔDi-OS [2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose], ΔDi-4S [2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose], and ΔDi-6S [2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose] were obtained from Seikagaku Kogyo Co., Tokyo; endo-β-N-acetylglucosaminidase F (EC 3.2.1) was from New England Nuclear, Boston, Massachusetts; L-(tosylamido-2-phenyl)ethyl chloromethyl ketone-trypsin (EC 3.4.21.4) (224 units/mg) was from Worthington Biochemical Co., New Jersey; Pronase E was from Kaken Kagaku Co., Tokyo; peroxidase conjugate antirat F(ab')$_2$ goat IgG was

from Cappel Laboratories, Inc., Cochranville, Pennsylvania; cellulose acetate membrane (Sepraphore III) was from Gelman Science Inc., Ann Arbor, Michigan; nitrocellulose membrane was from Bio-Rad Laboratories, California; cellulose-coated thin-layer chromatography (TLC) plate (Art. 5632, 10 × 10 cm) was from Merck, Darmstadt and Avicel SF (10 × 20 cm) from Asahi Kasei Kogyo Co., Tokyo; acrylamide was from Bio-Rad; Na[^{125}I] was from New England Nuclear; NaB[^{3}H]$_4$ (100 mCi/mmol) was from C.E.A., Gif-sur-Yvette, France; all other chemicals were of analytical grade from Nakarai Chemicals, Kyoto, Japan.

Isolation of PDS All procedures except density-gradient centrifugation were performed at 4°C. Neutral salt-insoluble newborn calf skin fractions (860 g wet weight), the residues of extraction with neutral salt as previously reported [10], were extracted with 17 liters of 4 M guanidinium chloride, 0.05 M Tris-HCl buffer, pH 7.5 in the presence of proteinase inhibitors (5 mM benzamidine hydrochloride, 25 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride, 2.5 mM N-ethylmaleimide, 2 mM *trans*-4-amino-methylcyclohexanecarboxylic acid, 10 μM L-*trans*-epoxysuccinic acid, 3.6 μM pepstatin). The suspension was centrifuged at 10,000 *g* for 30 min. The extract was dialyzed against 0.1 M NaCl, 6 M urea, 0.02 M sodium phosphate buffer, pH 7.0. Ion exchange chromatography was performed on DEAE-Sephadex A-50 (19 × 8 cm). The column was washed with 5 liters of 0.1 M NaCl, 6 M urea, 0.02 M sodium phosphate buffer, pH 7.0. The PGs were eluted with 2 M NaCl in the same buffer. Further purification was performed on a DEAE-Sephadex A-50 column (2.5 × 60 cm) with a linear gradient of 0.1–2.1 M NaCl in the above described buffer (total elution volume 2000 ml). The DS-containing fractions were subjected to affinity chromatography on a Con A-Sepharose 4B column (2.5 × 15 cm) after washing with 1 M NaCl, 0.2 M sodium phosphate buffer, pH 7.0. The bound proteins were eluted with the same buffer containing 0.1 M methyl α-D-mannoside. The Con A-bound and unbound fractions were dialyzed against water and freeze dried; redissolved in 0.5 M guanidinium chloride, 0.05 M sodium acetate buffer, pH 5.8; adjusted to a density of 1.36 g/ml and 1.4 g/ml, respectively, by addition of solid CsCl; and centrifuged at 100,000 *g* for 48 h at 15°C as previously reported [14]. Fractions of 3 ml were collected from the bottom and analyzed for uronic acid and protein. Uronic acid-rich fractions (density 1.33–1.47 g/ml from the Con A-bound and 1.34–1.48 g/ml from the unbound fraction) were pooled separately, dialyzed against water, and freeze-dried. The PGs that were obtained from the Con A-bound fraction were further purified by molecular sieve chromatography using a column of Sepharose CL-4B (0.9 × 51 cm) in 6 M guanidinium chloride, 0.05 M Tris-HCl buffer, pH 7.4. The PGs from the Con A-unbound fractions were also purified by column (2 × 90 cm) chromatography on Sepharose CL-4B in 1 M NaCl-0.05 M Tris-HCl buffer, pH 7.4, followed by a column (1.5 × 55 cm) of Ultrogel AcA-44 in 4 M guanidinium chloride-0.05 M Tris-HCl buffer, pH 7.4.

Analysis of Glycosaminoglycans Glycosaminoglycans which were obtained by β-elimination and pronase digestion from PGs were treated with chondroitinase ABC, chondroitinase AC, or *Streptomyces* hyaluronidase. They were also treated with $NaNO_2$ as previously described [15–17]. The resultant reaction mixtures were analyzed by 2-dimensional electrophoresis on cellulose acetate membranes [18]. For the determination of unsaturated disaccharide units, 200 μg of glycosaminoglycans obtained from the PGs, were digested with 150 milliunits of chondroitinase ABC or 100 milliunits of chondroitinase AC at 37°C for 8 h. After incubation, 30 to 60-μl aliquots (1 mg of glycosaminoglycan/ml) were subjected to chromatography on cellulose-coated TLC plates (10 × 20 cm) for the separation of unsaturated disaccharides as previously reported [19]. A mixture containing 20 μg each of authentic ΔDi-6S, ΔDi-4S, and ΔDi-0S was used as reference. The fluorescent spots were determined by high-speed TLC scanner (Shimadzu CS-920) at an absorbing wavelength of 232 nm. Uronic acid was determined by the carbazole method [20]. The amounts of glucuronosyl-galactosamine 4-sulfate and iduronosyl-galactosamine 4-sulfate in ΔDi-4S obtained by chondroitinase ABC or chondroitinase AC were quantified by the periodate/thiobarbiturate method [21]. The glycosaminoglycans (100 μg) or C4S (100 μg as a reference) were treated with 25 milliunits of chondroitinase ABC or 25 milliunits of chondroitinase AC for 16 h at 37°C.

Transblotting of Proteoglycans After sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, the PGs and the core proteins in the gel were transferred to nitrocellulose membrane in 25 mM Tris-192 mM glycine buffer, pH 8.3 containing 20% methanol (v/v), by electrophoresis at 7 V/cm for 3 h at 0°C as described by Towbin et al [22]. The membrane was stained with Alcian blue.

Characterization of Core Proteins Each PG was digested with chondroitinase ABC or chondroitinase AC in the presence of proteinase inhibitors as described by Oike et al [23]. The reaction mixture was subjected to SDS-polyacrylamide gel (10%, w/v) electrophoresis by the procedure of Laemmli [24]. The PGs and their core proteins were separately oxidized with periodate and reduced with NaB[^{3}H]$_4$ (100 μCi) as performed by Van Lenten and Ashwell [25]. Fluorography [26] was used for detection of the radioactivity. Core proteins of [^{3}H]PDS-H (high molecular-weight proteodermatan sulfate) were incubated with endo-β-N-acetylglucosaminidase H in 0.05 M citrate-phosphate buffer, pH 5.0 [27] or with endo-β-N-acetylglucosaminidase F in 100 mM sodium phosphate buffer, pH 6.1, containing 50 mM EDTA, 1% Nonidet P-40, 0.1% SDS, and 2-mercaptoethanol at 37°C for various times [28].

Analysis of Asparagine-Linked Oligosaccharides Low-molecular-weight proteoglycan containing dermaton sulfate and/or chondroitin sulfate (PGs-L) (8 mg) was dissolved in 0.4 ml of anhydrous hydrazine and heated at 100°C for 10 h. The oligosaccharides were reduced with 100 μmol of NaB[^{3}H]$_4$ in 200 μl of 0.05 M NaOH at 30°C for 4 h as previously described [29]. The labeled oligosaccharides were separated by high-voltage paper electrophoresis [30].

Tryptic Peptides Analysis After SDS-polyacrylamide gel electrophoresis of the PGs and their core proteins, Coomassie Brilliant Blue-stained bands were cut out from the gel and the proteins were radioiodinated with iodine-125 in the gel slice by modification of the chloramine T method [31]. Tryptic peptides were obtained by L-(tosylamido-2-phenyl) ethyl chloromethyl ketone-trypsin and analyzed on cellulose TLC plate by the method of Elder et al [32].

Amino Acid Analysis Amino acid analysis was performed in an autoanalyzer (Hitachi 835) after hydrolysis of PDS-H and PGs-L (500 μg each) in 6 M HCl at 110°C for 22 h.

Immunodetection Procedures The core proteins were transferred onto the nitrocellulose membrane from SDS-polyacrylamide slab gel [22]. The membrane was soaked in 10 mM Tris-HCl buffer, pH 7.5, containing 0.01% (v/v) Tween 20 and 0.25 M NaCl for 6 h at room temperature and incubated with antibodies against M_r = 55,000 core protein (diluted 1:200) for 16 h at 4°C. After washing with the above buffer for 6 h, the membrane was incubated with peroxidase-conjugated goat anti-rat-F (ab′)$_2$ serum (diluted 1:100) for 4 h at room temperature. The membrane was washed again with the same buffer at room temperature for 6 h and stained with 0.125% (w/v) 3,3′-diaminobenzidine tetrahydrochloride, 0.01% (v/v) H_2O_2 in the same buffer for 10 min.

Preparation of Antibody Against M_r = 55,000 Core Protein A rat was immunized using the M_r = 55,000 core protein from neutral salt-soluble PDS [10] as an immunogen. One milligram of keyhole limpet hemocyanin was added to 100 μg of

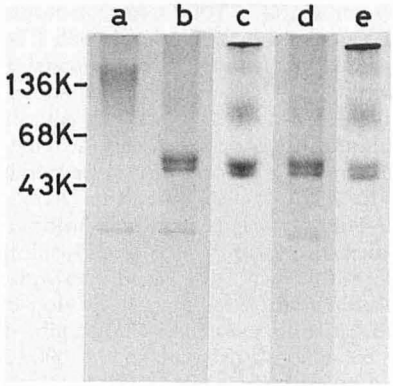

Figure 1. Sodium dodecyl sulfate-polyacrylamide slab gel (10%) electrophoretic pattern of PDS-H before (*a*) and after (*b*, *c*) treatment with chondroitinase ABC and further treatment with endo-β-N-acetylglucosaminidase H (*d*, *e*) stained with Coomassie Brilliant Blue (*a*, *b*, *d*) or by fluorography (*c*, *e*). PDS-H (50 μg dry weight or 27,000 cpm/50 μg) was treated with 20 milliunits of chondroitinase ABC in the presence of proteinase inhibitors at 37°C for 15 h. The protein-enriched core protein was further treated with 10 milliunits of endo-β-N-acetylglucosaminidase H at 37°C for 24 h. Positions of dimeric (*136K*) and monomeric (*68K*) bovine serum albumin and ovalbumin (*43K*) are indicated.

the M_r = 55,000 core protein in 1 ml of 145 mM NaCl, 2.8 mM NaH_2PO_4, 7.2 mM Na_2HPO_4 [phosphate-buffered saline (PBS), pH 7.2]; 0.5 ml of 0.25% (v/v) glutaraldehyde was added to this mixture, which was stirred for 24 h at 4°C and then dialyzed against PBS. The protein-hemocyanin conjugate was mixed with an equal volume of Freund's complete adjuvant. This mixture was injected into inguinal lymph nodes. Three weeks later, the rat was injected, as a booster, with about 75 μg of the protein conjugated to hemocyanin and mixed with Freund's incomplete adjuvant. Four additional boosters were injected into the enlarged lymph nodes at 2-week intervals. The serum was collected 1 week after the last booster. The IgG fraction from the serum was purified by chromatography on Protein A-coupled Sepharose 4B. The bound antibodies were eluted with 0.1 M glycyltyrosine in PBS and diluted to original volume with PBS.

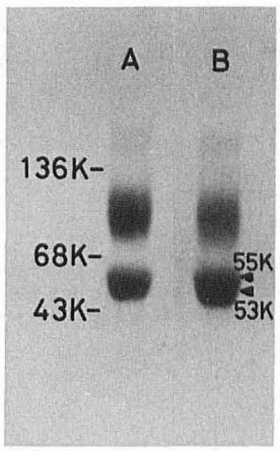

Figure 2. Sensitivity of ^3H-labeled core proteins toward endo-β-N-acetylglucosaminidase F. Partial purified PDS-H was labeled with $NaB[^3H]_4$ and the labeled core proteins were obtained by 70% ethanol precipitation followed by incubation with chondroitinase ABC. Gel electrophoresis was performed after treatment with 25 milliunits endo-β-N-acetylglucosaminidase F at 37°C for 24 h. Before (*A*) and after (*B*) treatment of endo-β-N-acetylglucosaminidase F. Markers are the same as shown in Fig 1.

RESULTS

Isolation and Characterization of PDS-H Proteoglycans containing DS could be separated into 2 fractions by Con A-Sepharose. Fifty-five percent of uronic acids obtained after DEAE ion exchange chromatography were recovered in the Con A-bound fraction. PDS-H was purified from this fraction by CsCl density-gradient centrifugation and by molecular sieve chromatography. About 20 mg of purified PDS-H were recovered from 860 g (wet weight) of neutral salt-insoluble calf skin. The glycosaminoglycans from PDS-H were detected as a single spot on

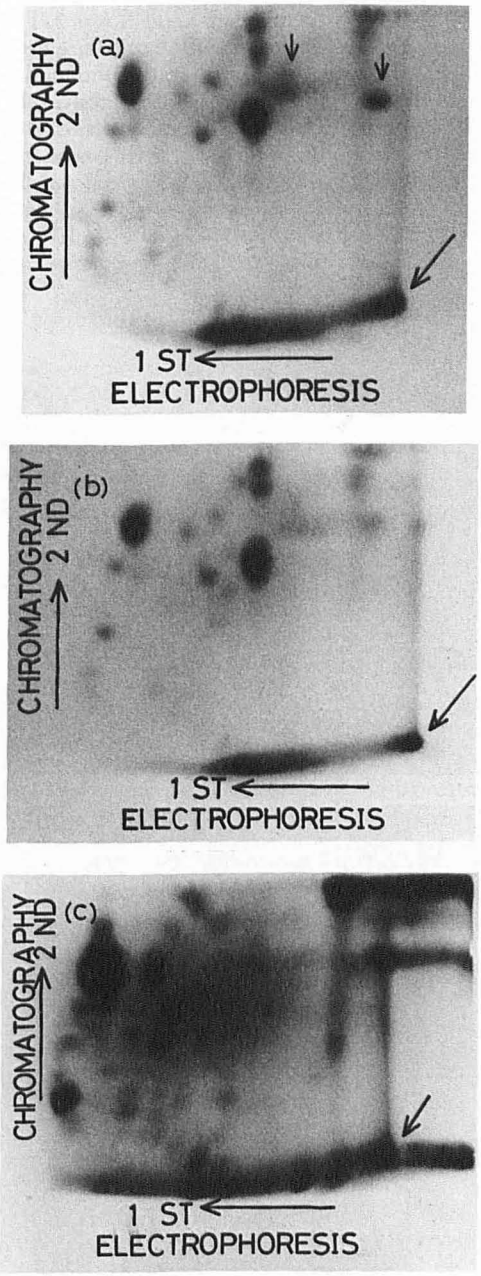

Figure 3. Tryptic peptide mapping of core proteins of PDS-H and PGs-L. Tryptic peptides (300,000 cpm) were electrophoresed in the first dimension on cellulose-coated TLC plates (10 × 10 cm) in acetic acid:formic acid:water (15:5:80) at 950 V for 12 min at 4°C and chromatographed in the second dimension in butanol:pyridine:acetic acid:water (32.5:25:5:20). Tryptic peptides of M_r = 55,000 core protein (*a*), M_r = 53,000 core protein of PDS-H (*b*), and M_r = 20,000 core protein from PGs-L (*c*). Some difference (*small arrows*) are shown in (*a*) in comparison with (*b*). *Large arrows* indicate the origin for electrophoresis.

acetate membrane. This component was resistant to chondroitinase AC but susceptible to chondroitinase ABC, giving 12% ΔDi-0S, 85% ΔDi-4S, and 3% ΔDi-6S. These uronosylhexosamine units in ΔDi-4S were calculated as 46% iduronosylhexosamine units and 54% glucuronsylhexosamine units from the periodate/thiobarbiturate method. The low iduronate content in composition of DS may reflect the age variation of the used sample. PDS-H migrated to the position of authentic dimeric bovine serum albumin as a broad-band by SDS-polyacrylamide gel (Fig 1*a*). The core proteins obtained by digestion with chondroitinase ABC were separated into 2 bands of M_r = 55,000 and M_r = 53,000 in a ratio of 1:1 from the staining intensity by Coomassie Brilliant Blue staining (Fig 1*b*). The ratio of radioactivity between M_r = 55,000 and M_r = 53,000 core protein was 3:1 (Fig 1*c*). In order to investigate the difference in molecular size between these 2 core proteins, they were further digested with endo-β-N-acetylglucosaminidase H for various periods of time. The ratio between the 2 core proteins determined by Coomassie Brilliant Blue staining and fluorogram did not change after enzymatic digestion (Fig 1*d*,*e*). As shown in Fig 2, little effect and only a slight reduction in molecular weight was observed after treatment of endo-β-N-acetylglucosaminidase F but M_r = 55,000 core protein did not convert to M_r = 53,000. The results indicate that both core proteins had N-linked oligosaccharide chains in their molecules but did not have high mannose type sugar chains, and M_r = 55,000 core protein is not the glycosylated form of M_r = 53,000. Tryptic peptide mapping on cellulose-coated TLC plates revealed that M_r = 55,000 and M_r = 53,000 core proteins were composed of mostly the same peptides (Fig 3). The result indicates that these core proteins were the same gene products.

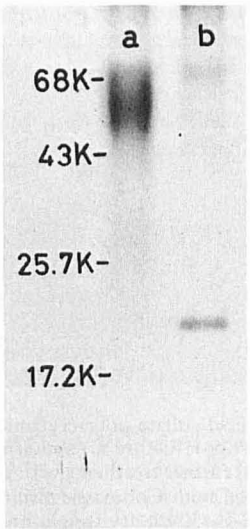

Figure 5. Fluorograms of radioactive proteoglycan and core protein from PGs-L. The PGs-L (18,000 dpm) was electrophoresed on SDS-polyacrylamide gel (10%) before (*a*) and after (*b*) treatment with chondroitinase ABC. Positions of monomeric bovine serum albumin (*68K*), ovalbumin (*43K*), chymotrypsinogen (*25.7K*), and myoglobin (*17.2K*) are indicated.

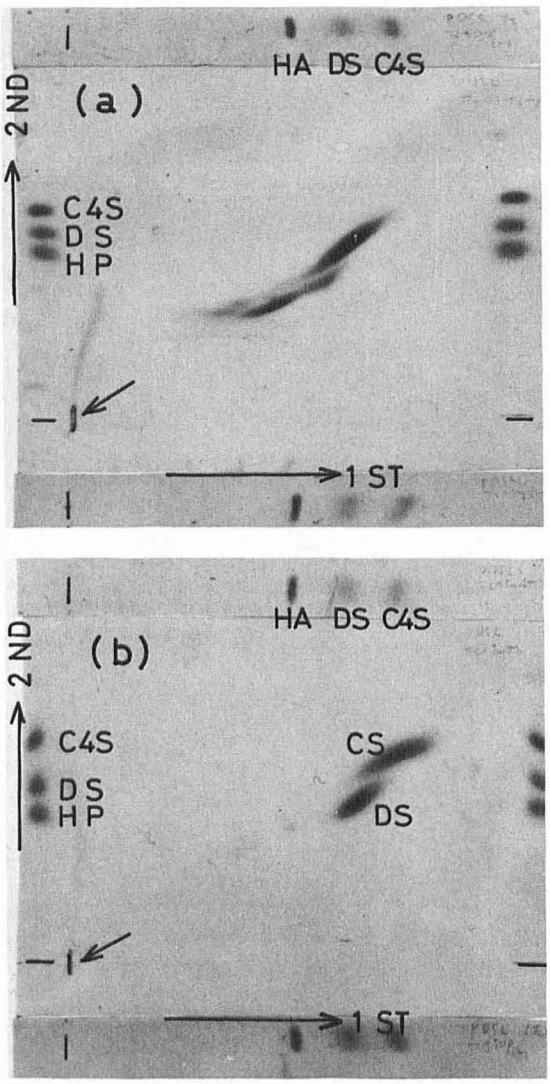

Figure 4. Two-dimensional electrophoretic pattern of PGs-L before (*a*) and after (*b*) β-elimination with NaOH. Electrophoresis was performed on cellulose acetate membrane using 0.1 M pyridine/0.47 M formic acid, pH 3.0, for the first dimension at 1 mA/cm for 90 min and 0.1 M barium acetate for the second dimension at 1 mA/cm for 3.5 h. Arrow indicates the origin for electrophoresis. Hyaluronic acid (HA), dermatan sulfate (DS), chondroitin 4-sulfate (C4S), heparin (HP).

Isolation and Characterization of PGs-L The PGs-L was recovered from the Con A-unbound fraction and migrated as 2 broad bands on a cellulose acetate membrane (Fig 4*a*). Dermatan sulfate and CS were detected as glycosaminoglycan components

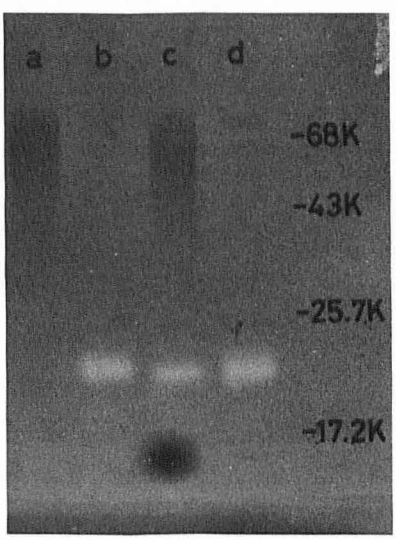

Figure 6. Transblotting profiles from SDS-polyacrylamide gel (10%) electrophoresis to a nitrocellulose membrane and staining with Alcian blue, before (*a*) and after (*b*) treatment with chondroitinase ABC, after treatment with chondroitinase AC (*c*), and further treatment of the sample in (*c*) with chondroitinase ABC (*d*).

after β-elimination with NaOH (Fig 4*b*). When PGs-L was centrifuged in CsCl density gradient, the ratio of DS to CS was heterogeneous depending on the fractions (data not shown). The combined fractions with density 1.34–1.48 g/ml were further applied on molecular sieve chromatography. About 5 mg of PGs-L were recovered from a Sepharose CL-4B column. The repeating unsaturated disaccharide units in PGs-L from the products of chondroitinase ABC were 15% ΔDi-6S, 55% ΔDi-4S, and 30% ΔDi-0S. The ratio of iduronosyl-galactosamine-4S to glucuronosyl-galactosamine-4S in glycosaminoglycan chains of PGs-L was 65:35. PGs-L showed a broad band from M_r = 50,000 to M_r = 70,000 by SDS-polyacrylamide gel electrophoresis. Its core protein obtained by digestion with chondroitinase ABC was assessed to be M_r = 20,000. When PGs-L was oxidized with periodate and reduced with NaB $[^3H]_4$ before digestion with chondroitinase ABC, the radioactivity was incorporated into the protein band (Fig 5). The PGs-L and its core protein obtained successive treatments with chondroitinases, were subjected to SDS-polyacrylamide gel electrophoresis, and then transferred to a nitrocellulose membrane. PGs-L but not the core protein was stainable with Alcian blue (Fig 6*a–d*). The products derived from chondroitinase AC treatment of PDS-L stayed at the position of intact molecule (Fig 6*c*). As shown in Table I, the amino acid composition of PGs-L was unique with high content of Gly, Pro, and Glx and low content of Leu and Lys in comparison with that described in the previous reports [10,33–36]. The tryptic peptide mapping of PGs-L was completely different from that of PDS-H (Fig 3). The core proteins from PDS-H reacted with the anti-M_r = 55,000 core protein, but the M_r = 20,000 core protein did not (Fig 7). In order to detect the presence of asparagine-linked oligosaccharide units, the products from hydrazinolysis of PGs-L were acetylated and reduced with NaB $[^3H]_4$. No radioactive N-acetylglucosaminitol could be found, indicating little of the asparagine-linked carbohydrate.

DISCUSSION

High-molecular-weight proteodermatan sulfate as a major proteoglycan was extracted with 4 *M* guanidinium chloride from neutral salt-insoluble materials of newborn calf skin. It comprised less than 0.5% of the dry weight of the tissue. As glycosaminoglycan components of the PDS-H, only DS chain was found. However, 2 different molecular size protein cores were found. One (M_r = 55,000) was found to be similar to the core protein extracted from neutral salt-soluble calf skin [10], and the other (M_r = 53,000) was not detected from the neutral salt-soluble fraction. Glössl et al [37] also observed the presence of 2 different core proteins (M_r = 48,000 and M_r = 45,000) of PDS from culture medium of human fibroblasts. The large-core protein had endo-β-N-acetylhexosamininidase H sensitive oligosaccharide chains [37]. The procedure using Con A-Sepharose 4B suggests that PDS-H has N-linked oligosaccharide chains in its core proteins. The core protein (M_r = 55,000) from neutral salt-soluble calf skin had di- and triantennary complex type oligosaccharide chains which were insensitive to endo-β-N-acetylglucosaminidase H [29], but susceptible to endo-β-N-acetylglucosaminidase F [28]. Proteodermatan sulfate from cultured human skin fibroblasts had high mannose oligosaccharide chains in large molecular size protein core (48K) and it was converted to small molecular size core protein (45K) by endo-β-N-acetylglucosaminidase H [37]. The M_r = 55,000 core protein from PDS-H was not converted to M_r = 53,000 core protein after incubation with endo-β-N-acetylglucosaminidase H or endo-β-N-acetylglucosaminidase F as shown in Figs 1 and 2. The tryptic peptide mapping of the 2 core proteins from PDS-H showed that these proteins have genetically the same amino acid sequences, although some peptides were distinguishable between these core proteins. These results indicate that the difference in molecular size between the 2 core proteins of PDS-H was not ascribed to difference of sugar chains but to the difference of peptide components. The M_r = 53,000 may have been derived from the M_r = 55,000 core protein by extracellular proteolytic processing.

The PGs-L have been isolated from PDS-H by affininty chromatography on Con A-Sepharose. They comprised about 20% of the PGs in guanidinium hydrochloride extracts. The PGs-L were separated into 2 different Alcian blue-positive spots on a cellulose acetate membrane. The glycosaminoglycan components were identified with DS and CS by electrophoretic mobility after β-elimination. The ratio of DS to CS in PGs-L showed heterogeneity in the fractions recovered following CsCl-density gradient centrifugation. These results suggest that PGs-L is composed of 2 different PGs, PDS and PCS. However, PGs-L migrated on SDS-polyacrylamide gel electrophoresis with a mobility of a protein with an apparent molecular weight of approximately 62,000. The size of core protein that was obtained by digestions with chondroitinase ABC was M_r = 20,000 in the same gel. About 50% of core proteins were liberated from the PGs-L by chondroitinase AC digestion (Fig 6*c*) while the remaining 50% of the PGs-L stayed at the same position of the intact PGs when transblotted to nitrocellulose membrane from SDS-polyacrylamide gel. No differences could be detected on peptide mappings between the core proteins obtained from chondroitinase AC or chondroitinase ABC treatment (data not shown). These results indicate that DS chain(s) and/or CS chain(s) bind to the M_r = 20,000 core protein. Recently low molecular size core protein of rat yolk sac tumor PG was isolated [38]. The glycosaminoglycan chains of the rat yolk sac tumor PG were composed of 10% DS and 90% CS [38] and the molecular weight of the core protein was calculated as 10,190 from CDNA [39]. PGs-L was composed of 30% iduronosylhexosamine and 70% glucuronosylhexosamine

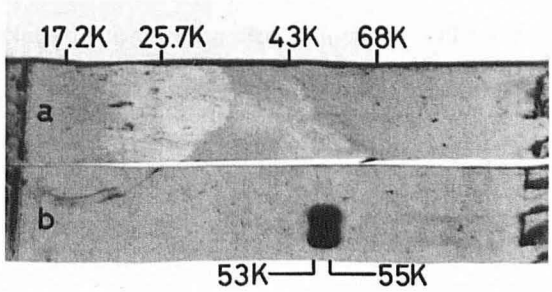

Figure 7. Immunologic analyses of core proteins from PGs-L (*a*) and PDS-H (*b*). Antibody against M_r = 55,000 core protein from proteodermatan sulfate was used. Markers are the same as shown in Fig 1.

Table I. Amino Acid Composition of Proteodermatan Sulfate of PDS-H and PGs-L Content (Residues/1000 Residues)

	PDS-L	PDS-H
Asx	105	131
Thr	47	45
Ser	82	71
Glx	168	104
Pro	105	76
Gly	121	73
Ala	56	51
½ Cys	11	10
Val	44	57
Met	11	10
Ile	51	79
Leu	70	129
Tyr		
Phe	22	35
Lys	57	77
His	24	26
Arg	25	27

Value for tyrosine was not obtained because it was obscured by the large galactosamine peak.

units. Several PGs containing core protein with N-linked oligosaccharide chains(s) were reported [29,33,40]. Such N-linked oligosaccharide chains could not be detected in PGs-L. The tryptic peptide mapping, amino acid composition, and immunologic nature of PGs-L were different from those of PDS-H. The structural difference obtained in the present study suggests that the novel low molecular PG may have a different function from the high molecular PGs.

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